Improving the compatibility of pesticides and predatory mites: recent findings on physiological and ecological selectivity

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Highlights

- New approaches have been proposed in the evaluation of pesticide selectivity towards natural enemies, in particular predatory mites.
- Predatory mites (i.e. phytoseiids) can become resistant to pesticides by mainly detoxification but also target-site mutations.
- Some recently developed acaricides show natural differential affinity for the target-site between spider mites and phytoseiids.
- Pesticide-free refuges and pesticide-treated nets increase the compatibility of pesticides with phytoseiids.
- Providing alternative foods can reduce the impact of pesticides on phytoseiids.

Abstract

Integrated pest management (IPM) greatly relies upon the application of selective pesticides that do not hinder the efficiency of biological control. Successful integration of chemical and biological control requires an in-depth consideration of both the physiological and ecological factors involved in this selectivity. Phytoseiid mites (Acari Phytoseiidae) represent an interesting case-study: they are amongst the most frequently used biological control agents and often are less affected by pesticides than their prey by natural tolerance or by developing resistance. The toxicity and selectivity of a pesticide is determined by physiological processes that include metabolism, transport, and the affinity to the target-site. Genomic and transcriptomic studies have started to elucidate the genetic and molecular mechanisms of differential toxicity in some phytoseiid species, such as mutation in the sodium channel conferring pyrethroid resistance. On the other side, ecological selectivity is achieved by smart applications of pesticides and management practices that influence the persistence of phytoseiid mites on plants. These include creating pesticide-free refuges, low application rates, bagging branches of insecticide-treated trees during spraying, and the use of treated nets. We further argue that although modern pesticides often show lower acute toxicity to phytoseiids and overall higher selectivity, there is a need for robust assays and procedures that quantify lethal and sublethal effects, through different routes and times of exposure.

Graphical abstract

Introduction

The compatibility between pesticides and biological control agents (BCA) is a major concern in agriculture, in particular in perennial crop systems where pest management programs can change rapidly, causing concerns for Integrated Pest Management (IPM) practitioners. In an attempt to reduce the impact on human health and the environment, broad-spectrum pesticides have been widely replaced by reduced-risk pesticides in many regions worldwide. This has led to an overall positive effect on IPM and conservation biological control [1–3]. However, even if the evolution to more selectivity is evident in modern crop protection chemistry, some of the reduced-risk insecticides are still harmful to BCA [4–6]. Also in organic crop systems, the selectivity of some natural pesticides proved to be lower than anticipated [7,8].

The aim to drastically reduce pesticide use is a priority in several countries, but many factors counteract this objective. For example, recent issues with invasive pests such as the brown marmorated stink bug *Halyomorpha halys* in America and Europe promoted insecticide use, disrupting IPM in fruit orchards [9]. Secondly, alternatives to fungicides, such as bio-pesticides are promising [10], but their implementation remains limited and extensive use of non-selective fungicides negatively affects BCA [1,11]. Very little is known whether other plant protection products (e.g., herbicides) may influence BCA [12].

Mesostigmatid predators (Acari Mesostigmata) are common in agricultural systems where they regulate population densities of arthropod and nematode pests. There is worldwide interest in the ecosystem services they provide and the identification of new biological control agents (BCA), and their use as alternatives to pesticides is still a hot topic [13]. The family Phytoseiidae is the most studied within the Mesostigmata (89% of papers using the keyword “predatory mites” in Scopus, URL: https://www.scopus.com) and a number of phytoseiid species are key-predators of spider mites, thrips, and whiteflies. Studies on phytoseiid biology, ecology, and behavior continue to increase in number. However, how pesticides affect phytoseiid persistence in crop systems and their efficiency to control pests remains poorly investigated.

Interestingly, several phytoseiid species became indicator species in eco-toxicological studies for regulatory purposes [14], but there is no agreement on how best to assess and evaluate pesticide effects on phytoseiids. Here, current trends in the evaluation of pesticide effects on phytoseiids are analyzed, together with factors determining the compatibility between pesticides and phytoseiids, focusing on ecological and physiological selectivity.

How to test pesticide effects on phytoseiids?

When evaluating the selectivity of pesticides on phytoseiids, laboratory tests are most often chosen due to the uncertainty of biotic and abiotic factors acting in the open field [15,16]. However, laboratory procedures do not reproduce the effects of the repeated application of pesticides in a growing season which commonly occurs with many products (e.g., fungicides). Field tests are considered more realistic for a variety of reasons: non-perfect coverage (and the

presence of refugia), the ability of the BCA to leave pesticide-treated areas and return, the decay of residues in the field (due to UV and other environmental factors), etc. Moreover, the application of a non-selective pesticide in field trials can induce an outbreak of secondary pests even during the trial, providing a clear picture of its impact on selected BCA and their functions [17]. Conversely, the availability of prey or alternative foods can alleviate the pesticide impact on phytoseiids independently of its selectivity [18]. Semi-field tests could represent a good compromise even if this approach has been seldom followed [19].

The evaluation of pesticide effects on phytoseiids evolved from assessing acute contact toxicity [20] to new protocols considering different life stages and effects on fecundity and fertility [21]. More recently, sublethal effects have also been considered with increasing interest as they influence reproduction, lifespan, response to sex pheromones, learning performance, searching behavior, and neurophysiology [22,23]. In addition, pesticides can act through multiple routes of exposure (direct or residual contact, food ingestion) and this was considered in studies on phytoseiids and their prey [24]. Applications of these concepts provided fundamental knowledge on the compatibility between pesticides and key-phytoseiids [25].

Procedures based on demographic parameters proved to be more effective than evaluations based on short-term mortality in estimating pesticide impacts on BCA [19]. They can estimate the effects that may occur in pesticide-exposed populations of pests and BCA over long periods and are currently widely adopted [26–29].

Whether the disruptive effects of pesticides on BCA seen in the laboratory can predict similar impacts in field conditions still remains an open question. In a recent study, the non-target effects of insecticides were studied in field trials by assessing their impact on selected natural enemies and the outbreaks of secondary pests [15]. The same insecticides had been previously tested in the laboratory. Authors tested two hypotheses: 1) pesticides found to be non-selective in laboratory bioassays will predict reductions in natural enemies in the field, and 2) these reductions will result in outbreaks of secondary pests. These hypotheses were only partially demonstrated because of many variables (e.g., field trial conditions, duration of the negative effects, plot size/inter-plot movement of BCA). Nevertheless, laboratory bioassays remain useful for developing IPM programs: pesticides showing severe effects over a great number of natural enemies were more frequently associated with pest outbreaks.

Generalizations across species phylogenetic groups are also to be interpreted carefully. For example, a recent study showed that acaricides marketed as selective for predatory mites (e.g. bifenazate, acequinocyl, cyflumetofen) are not selective for every phytoseiid species. Due to a high response variability between species within the same family, the authors concluded that there is a need to examine the non-target effects of pesticides for all key predator species individually [30].

Studies on the impact of pesticides on phytoseiids should be coupled with those on other BCA representatives of a crop, to create a comparative database predicting their harmful effects. Results obtained from several laboratory bioassays of acute mortality and life table response experiments were combined to estimate lethal and sublethal effects of several pesticides on seven

BCA, including a phytoseiid species, adopting stage-structured population models [16]. The aim was to extrapolate a single index that would predict the response of BCA populations to pesticides considering the life history parameters obtained from measurement of individuals in the bioassays and thus combining the “reduction coefficient approach” [21] and the “demographic approach” [16] into a single matrix model.

The final aims of laboratory, semi-field and field bioassays are to gain insights in the response of phytoseiids to pesticides and hereby provide crucial information for the design of IPM strategies. The information on the mechanisms involved in selectivity of predatory mites should be investigated using complementary approaches. Studying the mechanisms of physiological selectivity can inform on the rational development of pesticides with higher selectivity and lies at the basis for genetic and genomic improvement of biocontrol agents [31]

Physiological selectivity

Upon contact, the toxicity of a pesticide to phytoseiids is determined by key physiological processes, including penetration, activation, metabolism, transport, excretion and finally affinity for the target-site. These are usually referred to as toxicokinetic and toxicodynamic mechanisms [32] that can determine selectivity.

The metabolization of insecticides and acaricides is not only important as a major detoxification route, but also for the activation of pro-insecticides that are not toxic as such, but need *in vivo* metabolism [33]. Species-specific activation is most likely determining the selectivity of bifenazate, which has excellent activity on spider mites, but is harmless to most species of phytoseiids [34]. In addition, target-site selectivity can also determine selectivity, even when pesticides target very conserved processes. This was elegantly demonstrated for the recently developed acaricides cyflumetofen, cyenopyrafen and puflubumide, that target complex II of the mitochondrial electron transport chain. Concentrations of active metabolite inhibiting 50% of complex II activity differed more than 1000-fold between spider mites and some key pest insects species, and corresponded well with the observed differential toxicity. In addition, a 10- to 50-fold difference in affinity for the target-site was also observed between spider mites and phytoseiids [35–37].

Next to natural selectivity, phytoseiids can become resistant to pesticides, either by selection in the field, or by targeted laboratory selections. One of the first success stories was the use of organophosphate, carbamate and pyrethroid resistant lines of *Galendromus occidentalis* and *Typhlodromus pyr*i in orchards and vineyards in USA and EU respectively [1,19,38]. Resistance can have a profound effect on species composition in natural systems, as was recently shown in Japanese fruit orchards where the naturally occurring *Neoseiulus womersleyi* was replaced by more resistant *Neoseiulus californicus* [39]. Resistance evolves by changes in the toxicodynamic and toxicokinetic processes underlying toxicity, as described above [32, 409]. Most frequently, this entails increased detoxification or mutations in the target-site that alter binding kinetics, although a more complete variety of genetic mechanisms has been described recently [39]. For phytoseiids, resistance to etoxazole, hexythiazox, pyrethroids, and spirodiclofen has been associated with increased detoxification, as inferred from synergism tests and measuring detoxification enzymes

with model substrates [41–43]. However, potential target-site mediated resistance was not investigated in these studies, possibly by a lack of sequence data. Recently, genomic and transcriptomic studies have generated such genetic information for some phytoseiid species, which allows to identify and study both target-sites and detoxification genes. This will allow in the future to link resistance with specific genes, more than overall general processes like oxidation and hydrolysis [44*–47]. In addition, some clear cases of target-site resistance have been described. High resistance levels to chlorpyrifos in *Kampimodromus aberrans* were linked with a substitution, F331W, in the acetylcholinesterase [48*]. Recently, resistance to pyrethroids in *Phytoseiulus persimilis* and *Amblyseius swirskii* has been associated with mutations in the voltage gated sodium channel (L Benavent-Albarracin *et al.*, abstract, Resistance 2019, Harpenden UK, September 2019). However, in comparison with pests, the mechanisms of resistance have been only poorly understood in phytoseiids, and many cases of resistance, such as abamectin resistance in *Amblyseius longispinosus* await elucidation [49].

Selectivity of pesticides can most likely be designed more strongly by better understanding the combination of several synergistic physiological processes that determine toxicity, and should be a prime focus if we are to develop more selective compounds. The era of 'omics' will most likely be a strong impetus for a more fundamental understanding, both of natural toxicity, as well as understanding the evolution of resistance.

Ecological selectivity and management practices that enhance compatibility

Biological control tactics that are based on ecological selectivity might be exploited to improve the use of compounds that are not physiologically selective but crucial in plant protection. Ecological selectivity can be achieved by limiting the exposure of a BCA to pesticides in time and space [20]. The temporal separation is a primary aspect, and laboratory evaluations have considered the effects of exposure to aged residues under realistic conditions [50,51]. In addition, spatial separation between pesticides and phytoseiids can be promoted at different scales. At the plant scale, the separation can be obtained by leaving untreated areas within the plants. Irritability caused by acaricides may favor the escape of phytoseiids from contaminated surfaces. On coconut, phytoseiids avoided acaricide contaminated areas, and the highest repellency seemed to be associated with the least selective products [52]. Localized applications of pesticides can influence within plant distribution of mites and their dispersal at higher scales induced by wind take-off [53**]. Survival and efficiency in biological control of dispersing mites are linked to the physiological selectivity of the pesticides used [52,54,55]. Kakoki et al. [56*] proposed the use of low dose partial spraying (i.e. applying sub-lethal dosages of pesticides to the leaf layer) and this resulted in a higher persistence of phytoseiids as compared to full coverage sprays. Pesticide-free refuges can also be created by bagging the branches of insecticide-treated trees during spraying. Similar refuges have been established using slow-release sachets of phytoseiids protected by plant-attached shelters [57]. Reduction in routes of exposure can promote the compatibility between pesticides and phytoseiids. Phytoseiids consuming prey that was systemically contaminated by drench application of thiamethoxam, were less affected as compared to those exposed to multiple routes [24].

Pesticide-treated nets avoid the direct application of pesticides to the crop and predators [58,59], but information on their impact on BCA is limited. The use of bifenthrin-treated nets was compatible with phytoseiid releases reducing pest densities [60*]. Laboratory exposure to the insecticide-treated net resulted in only moderate toxicity to phytoseiids despite the poor selectivity of compounds. In Africa, acaricide-treated nets combined with phytoseiid releases provided better control of spider mites compared to the two techniques separately [61*]. A higher spider mites mortality was induced by the combination of nets and predation upon eggs.

Off-field habitats represent a reservoir for phytoseiid populations that can potentially colonize crops [62,63]; thus compatibility between pesticides and phytoseiids could be promoted by reducing pesticide drift. Low-drift nozzles and anti-drift adjuvants were effective in controlling key pests in orchards and vineyards, while they did not affect phytoseiids [64].

Crop management options that increase the availability of alternative foods are important for generalist phytoseiids [65,66]. Pollen provisioning reduced the impact of insecticides on phytoseiids occurring on apple, alleviating sub-lethal effects with favorable consequences at population level [67]. Another study on grapevine, found a correlation between phytoseiid populations and foliar symptoms of downy mildew in different plots treated with fungicides: the availability of downy mildew as alternative food reduced the impact of some fungicides on beneficial mites [18].

Conclusions and future prospects

The use of pesticides has changed and continues to change today, as products currently applied in various regions in the world have often a lower acute toxicity and improved selectivity compared to those applied in the past. Characterizing the variety of sublethal side-effects makes it possible to delineate the eco-toxicological profile of a pesticide, but this remains challenging. Repellency or reduced prey consumption can be an explanation for the poor correspondence between predator densities and biological control outcome [6]. New routes of exposure have recently been integrated in toxicity testing [68] and special attention to the effects of sublethal exposure has increased our understanding of pesticide predator interactions [69]. Pesticide effects on life history can change over time according to their persistence, which influences BCA populations. Finally, indirect impacts of pesticides or their combination are often not considered in life table response experiments, adding uncertainty to predictions [16]. In addition, generalization of selectivity across species should be avoided [30]. Summarizing, it is clear that advanced toxicological studies coupled with insights on the physiological selectivity are needed to favor tactics based on ecological selectivity. Such an approach should also be followed to manage other BCA of economic importance in various agricultural systems that show a potential in biological control [70-72].

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